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International application number: PCT/EP05/002015

International filing date: 25 February 2005 (25.02.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/548,581
Filing date: 27 February 2004 (27.02.2004)

Date of receipt at the International Bureau: 12 April 2005 (12.04.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

PA 1253719

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APPLICATION NUMBER: ***60/548,581***
FILING DATE: ***February 27, 2004***

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

P. SWAIN
Certifying Officer

17236 U.S.PTO 022704

Docket Number	4-33658P1

FILING BY "EXPRESS MAIL" UNDER 37 CFR 1.10

EL997254711US	February 27, 2004
Express Mail Label Number	Date of Deposit

Address to: **MS: Provisional Patent Application**
Commissioner for Patents.
PO Box 1450
Alexandria, VA 22313-1450

19587 U.S.PTO 60/548581 022704

PATENT COVER SHEET FOR PROVISIONAL APPLICATION

Transmitted herewith for filing under 37 CFR §1.53(c) is the PROVISIONAL APPLICATION for patent of

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TITLE OF THE INVENTION (280 characters max)

ORGANIC COMPOUNDS

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ENCLOSED APPLICATION PARTS *(check all that apply)*

- ☒ Specification (Including Any Claims and Abstract) - 13 pages
- ☒ Drawings - 2 sheets
- ☒ Other (specify): Application Data Sheet

METHOD OF PAYMENT

The Commissioner is hereby authorized to charge filing fee and any additional fees required to Deposit Account Number: 19-0134 in the name of Novartis.	PROVISIONAL FILING FEE AMOUNT: $ 160

☐ U.S. Government agency and contract number: (if the invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.)

Respectfully submitted,

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APPLICATION INFORMATION

Title Line One:: ORGANIC COMPOUNDS
Total Drawing Sheets:: 2
Formal Drawings?:: No
Application Type:: Provisional
Docket Number:: 4-33658P1
Secrecy Order in Parent Appl.?:: No

REPRESENTATIVE INFORMATION

Representative Customer Number:: 1095

Source:: PrintEFS Version 2.0

Organic Compounds

The present invention relates to organic compounds, e.g. to an assay for identifying an agent that modulates the interaction of interleukin-23 or interleukin-12 with a corresponding receptor thereof.

It is known from the literature that interleukin-23 and interleukin-12 play an important role as mediators, e.g. in the immune system, see e.g. Puccetti P. et al., Crit.Rev.Immunol.2002, 22 (5-6), 373-90, in infectious diseases, see e.g. Holscher C. et al, J.Immunol. 2001, 167(12)6957-66 and in inflammation, see e.g. Lupusoru C.E. et al., Rev.Med.Chir.Soc. Med.Nat.Iasi, 2002, 106(1), 24-9.

In one aspect the present invention provides an assay for identifying an agent that modulates the interaction of interleukin-23 or interleukin-12 with a corresponding receptor thereof comprising

a) contacting interleukin-23 or interleukin-12 with a corresponding interleukin receptor or a part thereof in the absence and in the presence of a candidate compound which is expected to modulate the interaction of said interleukin with said receptor for a sufficient period of time so that a complex between said interleukin and said receptor can be formed,

b) optionally separating the complex from uncomplexed fractions,

c) detecting the complex formed in step a),

d) determining whether there is a difference in the amount of complex formed in case a candidate compound was absent or present in step a), and

e) choosing a candidate compound where a difference is determined in step d) as an agent,

e.g. the receptor is the interleukin-23 p19 receptor or the interleukin-12 p40 receptor or a part thereof, e.g. a receptor as described by Parham Ch. et al., Journal of Immunology, 2002, 168:5699-5708.

Such receptor includes a wild-type receptor for interleukin-23 and/or interleukin 12 or a part thereof. "A part thereof" as used herein is understood to be a modified or mutated Interleukin-23 or interleukin-12 receptor, which retains its specificity for interleukin-23 and/or interleukin-12. E.g. the receptor is a molecule, such as a protein, which is smaller than the

wild type receptor, e.g. a receptor protein having less amino acids than the wild type receptor protein, or a molecule having a modification (mutation), e.g. having a substitution or an addition of a nucleotide or an amino acid as compared to the wild type receptor, but still retaining its specificity for interleukin-23 and/or interleukin-12.

In another aspect the present invention provides an assay for identifying an agent that modulates the interaction of interleukin-23 or interleukin-12 with a corresponding receptor wherein the receptor or a part thereof is fused to an immunoglobulin or a fragment thereof.

A fragment of an immunoglobulin is e.g. the constant region (Fc) part of an immunoglobulin, e.g. immunoglobulin G, e.g. an interleukin-23 receptor/Fc fusion protein or an interleukin-12 β1/Fc fusion protein.

Optionally a complex formed can be separated from uncomplexed fractions.
In case the complex formation reaction is carried out as a homogenous reaction in solution the separation can be carried out according, e.g. analogously, to methods as conventional, e.g. chromatographically, e.g. size exclusion chromatography.
In case the complex formation reaction is carried out as a heterogenic reaction on a solid phase, the complex can be separated according, e.g. analogously, to methods as conventional, e.g. by washing the solid phase to which the complex formed is bound, e.g. by use of appropriate washing solutions.

For detecting the complex formed detection means may be used. Such detection means include those as conventional in the field of immunoassays, e.g. enzyme linked immunoassays (ELISAs). Detection means used in the present invention comprise molecules which recognize interleukin-23 and/or interleukin-12, e.g. a molecule which is directly or indirectly detectable. Detection means of the present invention preferably comprise an antibody, e.g. an antibody which recognizes interleukin23 and/or interleukin-12, e.g. a label bearing interleukin-12 antibody.
The label may be one as conventional, e.g. biotin or an enzyme such as alkaline phosphatase (AP), horse radish peroxidase (HRP) or peroxidase (POD) or a fluorescent molecule, e.g. a fluorescent dye. Preferably the label is biotin. The label bearing molecule, e.g. the label bearing antibody, may be detected according to methods as conventional, e.g. via fluorescence measurement or enzyme detection methods.

Optionally the receptor, the receptor fused to an immunoglobulin or a fragment thereof or the detectable molecule comprised in the detection means is immobilized on a solid phase. An appropriate solid phase includes e.g. one as conventional, e.g. a plastic plate like a polystyrene or polyvinyl plate, especially a microtiter plate. Also microbeads can be used as a solid phase, e.g. coated microbeads. The solid phase can be coated with a coating material the nature of which depends e.g. on the label comprised in the detection means. The coating material should be able to bind to the label, e.g. the label is biotin and the coating material includes streptavidin, e.g. covalently bound to the solid phase.

In a preferred aspect the interleukin receptor, e.g. the interleukin receptor/Fc fusion protein, is immobilized on a solid phase, e.g. on microtiter plates, and after incubation with the corresponding interleukin and optionally separating the complex formed from uncomplexed fractions, e.g. by washing the solid phase with an appropriate washing solution. The complex formed on the solid phase, e.g. on microtiter plates, may be detected with detection means comprising a biotin-labeled anti-interleukin-12 antibody, strepatvidin-alkaline phosphatase and a phosphatase substrate and measuring the absorbance at a defined wavelength, e.g. at 405nm.

A candidate compound includes compound(s)(libraries) from which its modulating effect on the interaction of interleukin-23 or interleukin-12 with a corresponding receptor thereof can be determined. Compound (libraries) include for example oligopeptides, polypeptides, proteins, antibodies, mimetics, small molecules, e.g. low molecular weight compounds (LMW`s).

An agent is a compound which influences (inhibits) the binding of interleukin-23 or interleukin-12 to a corresponding receptor thereof as detected/determined in step d) in an assay provided by the present invention.

An agent is one of the chosen candidate compounds and may include oligopeptides, polypeptides, proteins, antibodies, mimetics, small molecules, e.g. low molecular weight compounds (LMW`s). An agent includes one or more agents, e.g. a combination of agents.

In another aspect the present invention provides an assay for identifying an agent that modulates the interaction of interleukin-23 with a corresponding receptor thereof comprising a) contacting interleukin-23 with the interleukin-23 p19 receptor, the interleukin-12 p40

receptor or a part thereof in the absence and in the presence of a candidate compound which is expected to modulate the interaction of said interleukin with said receptor for a sufficient period of time so that a complex between said interleukin and said receptor can be formed,

b) optionally separating the complex form uncomplexed fractions,

c) detecting the amount of complex formed in step a),

d) determining whether there is a difference in the amount of complex formed in case a candidate compound was absent or present in step a), and

e) choosing a candidate compound where a difference is determined in step d) as an agent,

e.g. the detection means for detecting a complex formed between interleukin-23 and the interleukin-23 p19 receptor, the interleukin-12 p40 receptor or a part thereof comprises a label bearing, e.g. biotinylated, interleukin-12 antibody.

In another aspect the present invention provides an assay for identifying an agent that modulates the interaction of interleukin-12 with a corresponding receptor thereof comprising

a) contacting interleukin-12 with the interleukin-12 p40 receptor or a part thereof in the absence and in the presence of a candidate compound which is expected to modulate the interaction of said interleukin with said receptor for a sufficient period of time so that a complex between said interleukin and said receptor can be formed,

b) optionally separating the complex form uncomplexed fractions,

c) detecting the complex formed in step a),

d) determining whether there is a difference in the amount of complex formed in case a candidate compound was absent or present in step a), and

e) choosing a candidate compound where a difference is determined in step d) as an agent,

e.g. the detection means for detecting a complex formed between interleukin-12 and the interleukin-12 p40 receptor or a part thereof comprises a label bearing, e.g. biotinylated, interleukin-12 antibody.

In another aspect the present invention provides a kit for identifying an agent that modulates the interaction of interleukin-23 and/or interleukin-12 with a corresponding receptor comprising

a) interleukin-23 and/or interleukin-12,

b) the interleukin-23 p19 receptor and/or the interleukin-12 p40 receptor and/or or a part thereof,

c) optionally detection means,

d) instructions for use of said kit, and
e) optionally a solid phase.

In another aspect the present invention provides a kit as provided by the present invention, wherein
- said detection means comprise a label bearing, e.g. biotinylated, interleukin-12 antibody,
- the interleukin receptor or part thereof is fused to an immunoglobulin or a fragment thereof, e.g. an interleukin-23 receptor/Fc fusion protein or an interleukin-12 receptor β1/Fc fusion protein.

In another aspect the present invention provides a kit for identifying an agent that modulates the interaction of interleukin-23 with a corresponding receptor comprising
a) interleukin-23,
b) the interleukin-23 p19 receptor and/or the interleukin-12 p40 receptor or a part thereof,
c) optionally detection means,
d) instructions for use of said kit, and
e) optionally a solid phase.

In another aspect the present invention provides a kit for identifying an agent that modulates the interaction of interleukin-12 with a corresponding receptor comprising
a) interleukin-12,
b) the interleukin-12 p40 receptor or or a part thereof,
c) optionally detection means,
d) instructions for use of aisd kit, and
e) optionally a solid phase.

Such kit as provided by the present invention may further comprise a substantial component including an appropriate environment of a sample to be tested and, e.g. appropriate means to determine the effect of a candidate compound in a sample to be tested.

In another aspect the present invention provides an agent identified by an assay of the present invention.

In another aspect the present invention provides the use of an agent of the present invention as a pharmaceutical.

In another aspect the present invention provides the use of an agent of the present invention for the manufacture of a medicament for the treatment of autoimmune related diseases, including allergic diseases, inflammatory diseases and infectious diseases.

In another aspect the present invention provides a pharmaceutical composition comprising an agent of the present invention beside at least one pharmaceutical excipient, e.g. appropriate carrier and/or diluent, e.g. including fillers, binders, disintegrators, flow conditioners, lubricants, sugars and sweeteners, fragrances, preservatives, stabilizers, wetting agents and/or emulsifiers, solubilizers, salts for regulating osmotic pressure and/or buffers.

In another aspect the present invention provides a pharmaceutical composition according to the present invention, further comprising another pharmaceutically active agent.

Such compositions may be manufactured according, e.g. analogously to a method as conventional, e.g. by mixing, granulating, coating, dissolving or lyophilizing processes. Unit dosage forms may contain, for example, from about 0.5 mg to about 1000 mg, such as 1 mg to about 500 mg.

In another aspect the present invention provides the use of the interleukin-23 p19 receptor, the interleukin-12 p40 receptor or a part thereof for identifying an agent that modulates the interaction of interleukin-23 with one of said receptors or parts thereof.

In another aspect the present invention provides the use of an interleukin-12 p40 receptor or a part thereof for identifying an agent that modulates the interaction of interleukin-12 with said receptor or a part thereof.

In another aspect the present invention provides a method for determining whether a receptor is specific for interleukin-23 or interleukin-12 or both or none comprising
a) providing a receptor or a part thereof,
b) contacting interleukin-23 with the receptor of step a) for a sufficient period of time so

that a complex between said interleukin and said receptor can be formed,

c) contacting interleukin-12 with the receptor of step a) for a sufficient period of time so that a complex between said interleukin and said receptor can be formed,

d) optionally separating the complex formed in step b) and/or c) from uncomplexed fractions,

e) detecting the complex formed in step b) and/or in step c) with detection means,

f) determining whether the receptor is

- specific for interleukin-23, which is the case if
  a complex formation of step b) and
  no complex formation of step c) is detected, or
- specific for interleukin-12, which is the case if
  a complex formation of step c) and
  no complex formation of step b) is detected, or
- specific for both interleukin-23 and interleukin-12, which is the case if
  a complex formation of step b), and
  a complex formation of step c) is detected, or
- unspecific for interleukin-23 and interleukin-12, which is the case if
  no complex formation of step b), and
  no complex formation of step c) is detected.

Description of the figures:

Figure 1 shows the concentration dependent binding curve of interleukin-23 to the interleukin-23 receptor, wherein the complex formed is detected with detection means comprising a biotinylated anti-interleukin-12 antibody, avidin and alkaline phosphatase substrate reagent. The absorbance at 405nm (OD405) is measured.

Figure 2 shows the concentration dependent binding curve of interleukin-23 to the interleukin-12 receptor β1, wherein the complex formed is detected with detection means comprising a biotinylated anti-interleukin-12 antibody, avidin and alkaline phosphatase substrate reagent. The absorbance at 405nm (OD405) is measured.

In the following examples all temperatures are in degree centigrade and are uncorrected.

The following ABBREVIATIONS are used:

BSA bovine serum albumin

Fc	constant region of immunoglobulin G
PBS	phosphate buffered saline
RT	room temperature

EXAMPLES:

Example 1:

IL-23 receptor binding assay

A fusion protein comprising IL-23 receptor and Fc (R&D Systems #1400-IR9) is coated onto 96-well plates (Nunc Maxisorb #442404) at a concentration of 1 µg/ml in PBS, pH 7.4, 100 µl/well. All incubation steps are carried out at RT in a humidified chamber overnight. The plates are emtied and filled with 200 µl/well of SuperBlock (Pierce #37535). After 1 hour, the blocking reagent is discarded. 100 µl/well of IL-23 (R&D Systems #1290-IL) are added in triplicate at different concentrations in assay diluent comprising 20 mM Tris-HCl, 150 mM NaCl, 0,1% of BSA, 0.05% of Tween20 in PBS, pH 7.4. for 1.5 hours. The plates are washed 4 times with wash buffer (0.05% Tweeen 20 in PBS, pH7.4). 100 µl/well of a biotinylated goat anti-IL-12 antibody (R&D Systems #BAF219) at a concentration of 250 ng/ml in assay buffer are added for 1.5 hours. After washing 4 times with wash buffer, the plates are incubated with 50 µl/well of ExtraAvidin (Sigma #E-2636) diluted 1 : 2000 in SuperBlock. After 1.5 hours, the plates re washed 4 times with wash buffer and 100 µl/well of alkaline phosphatase substrate reagent (BioRad #172-1063) are added. Color development is stopped by addition of 50 µl/well of 2N NaOH. The absorbance is read on a SLT microtiter plate reader at 405 nm with a reference wavelength of 690 nm.

Results are shown in Figure 1.

Example 2:

IL-12 receptor β1 binding assay

The assay is carried out as described in example 1 but using the IL-12 receptor β1/Fc fusion protein (R&D Systems #839-B1). Results are shown in Figure 2.

Patent claims

1. Assay for identifying an agent that modulates the interaction of interleukin-23 or interleukin-12 with a corresponding receptor thereof comprising
   a) contacting interleukin-23 or interleukin-12 with a corresponding interleukin receptor or a part thereof in the absence and in the presence of a candidate compound which is expected to modulate the interaction of said interleukin with said receptor for a sufficient period of time so that a complex between said interleukin and said receptor can be formed,
   b) optionally separating the complex from uncomplexed fractions,
   c) detecting the complex formed in step a),
   d) determining whether there is a difference in the amount of complex formed in case a candidate compound was absent or present in step a), and
   e) choosing a candidate compound where a difference is determined in step d) as an agent.

2. The assay of claim 1, wherein the receptor is the interleukin-23 p19 receptor or the interleukin-12 p40 receptor or a part thereof.

3. The assay of any one of claims 1 or 2, wherein the receptor or a part thereof is fused to an immunoglobulin or a fragment thereof.

4. The assay of any one of claims 1 to 3, wherein
   - the interleukin is interleukin-23,
   - the receptor is the interleukin-23 p19 receptor, the interleukin-12 p40 receptor or a part thereof.

5. Assay of any one of claims 1 to 3, wherein
   - the interleukin is interleukin-12,
   - the receptor is the interleukin-12 p40 receptor or a part thereof.

6. Kit for identifying an agent that modulates the interaction of interleukin-23 and/or interleukin-12 with a corresponding receptor comprising
   a) interleukin-23 and/or interleukin-12,

b) the interleukin-23 p19 receptor and/or the interleukin-12 p40 receptor and/or or a part thereof,

c) optionally detection means,

d) instructions for use of said kit, and

e) optionally a solid phase.

8. The kit of claim 7, wherein said detection means comprise a label bearing interleukin-12 antibody.

9. The kit of any one of claims 7 or 8, wherein the interleukin receptor or part thereof is fused to an immunoglobulin or a fragment thereof.

10. An agent identified by an assay of any one of claims 1 to 5.

11. Use of an agent of claim 10 as a pharmaceutical.

12. Use of an agent of claim 10 for the manufacture of a medicament for the treatment of autoimmune related diseases, inflammatory diseases and infectious diseases.

13. Pharmaceutical composition comprising an agent of claim 10 beside at least one pharmaceutical excipient.

14. Use of the interleukin-23 p19 receptor, the interleukin-12 p40 receptor or a part thereof for identifying an agent that modulates the interaction of interleukin-23 with one of said receptors.

15. Method for determining whether a receptor is specific for interleukin-23 or interleukin-12 or both or none comprising

a) providing a receptor or a part thereof,

b) contacting interleukin-23 with the receptor of step a) for a sufficient period of time so that a complex between said interleukin and said receptor can be formed,

c) contacting interleukin-12 with the receptor of step a) for a sufficient period of time so that a complex between said interleukin and said receptor can be formed,

d) optionally separating the complex formed in step b) and/or c) from uncomplexed

fractions,

e) detecting the complex formed in step b) and/or in step c) with detection means,

f) determining whether the receptor is

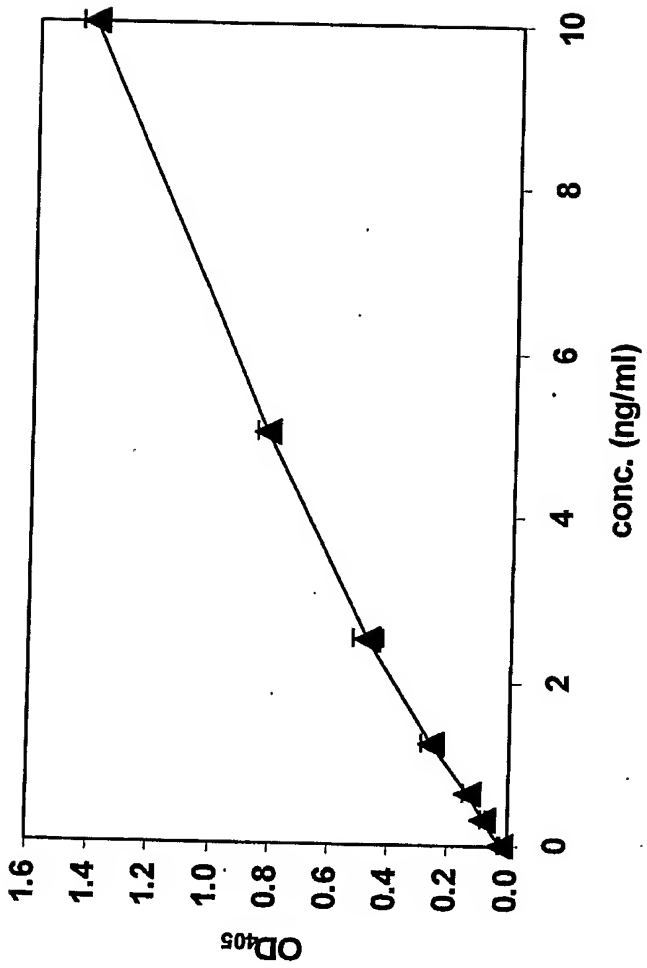
- specific for interleukin-23, which is the case if
  a complex formation of step b) and
  no complex formation of step c) is detected, or
- specific for interleukin-12, which is the case if
  a complex formation of step c) and
  no complex formation of step b) is detected, or
- specific for both interleukin-23 and interleukin-12, which is the case if
  a complex formation of step b), and
  a complex formation of step c) is detected, or
- unspecific for interleukin-23 and interleukin-12, which is the case if
  no complex formation of step b), and
  no complex formation of step c) is detected.

IL/27-Feb-2004

Abstract

The invention relates to organic compounds, e.g. to an assay for identifying an agent that modulates the interaction of interleukin-23 or interleukin-12 with a corresponding receptor.

Figure 1



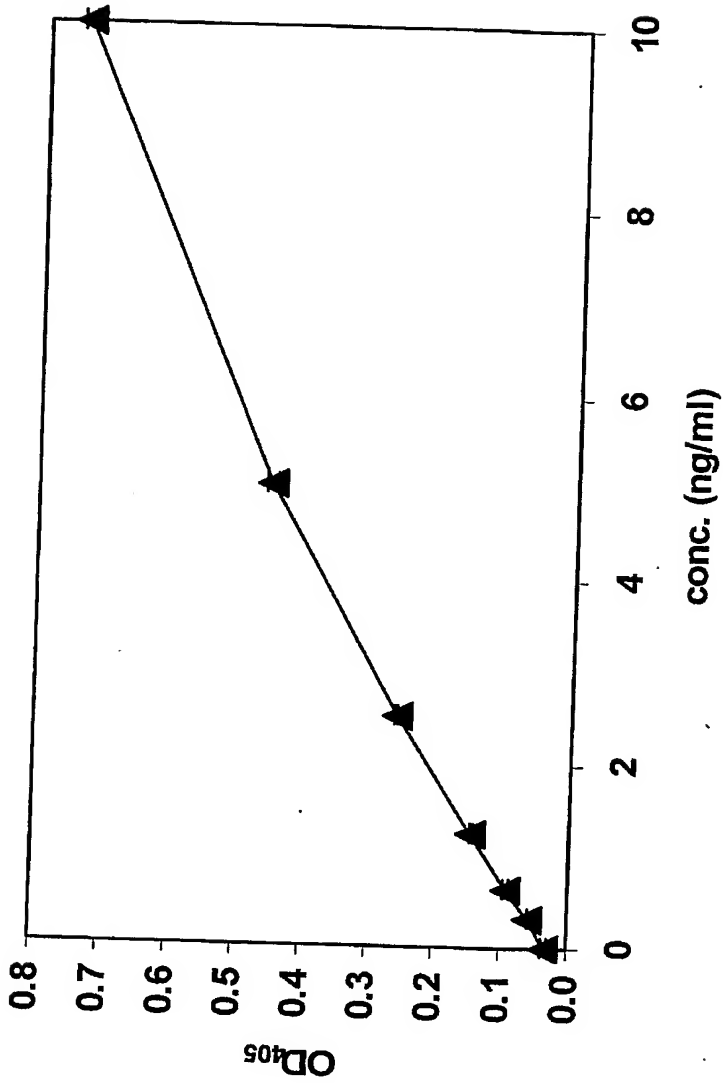


Figure 2